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Title: Chemical Communication and Mother-Infant Recognition.

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Abstract: Fifty years after the term ‘pheromone’ was coined by Peter Karlson and Martin Lüscher, the search for these semiochemicals is still an elusive goal of chemical ecology and communication studies. Contrary to what appears in the popular press, the race is still on to capture and define human scents. Over the last several years, it became increasingly clear that pheromone-like chemical signals probably play a role in offspring identification and mother recognition. Recently, we analyzed the volatile compounds in sweat patch samples collected from the para-axillary and nipple-areola regions of women during pregnancy and after childbirth. We hypothesized that, at the time of birth and during the first weeks of life, the distinctive olfactory pattern of the para-axillary area is probably useful to newborns for recognizing and distinguishing their own mother, whereas the characteristic pattern of the nipple-areola region is probably useful as a guide to nourishment.

Pheromones or signature odors? Throughout the lives of most mammalian species, the sense of smell plays an important role in response to chemical messengers involved in different behaviors. Within the overall olfactory communication pheromones are very important actors. The term ‘pheromone’ –referred to the chemical compounds used to communicate between individuals of the same species –was coined by

Peter Karlson and Martin Lüscher in 1959 ¹. Over the last 50 years, pheromones, sending messages between individuals, have been found in many species across the animal kingdom.

The so-called “individual odors”, learned for recognition, do not seem to fit Karlson and Lüscher’s definition. In the past, some researchers even doubted that humans could have their behavior altered by something as simple as an instinctive reaction to smell. Now, after years of debate, it seems clear that these variable odors are not pheromones and instead are better referred to as ‘signature odors’.

But species-specific small molecules, that fit the classic pheromone definition, have now been identified for mammals. It appears clear that signature odors and pheromones can be mixed for effect. Some mammals, including elephants and mice, present their small-molecule pheromones in the cleft of highly variable lipocalin proteins. Pheromone signals can also be overlaid and improved with individual signature odors, the proteins release the small molecules slowly, making them last longer.

The consensus now is that the human use of pheromones, as in all mammals, appears likely. The armpits are prime source candidates, as their odor emanation develops along with other changes at puberty. However, both human behavior and chemical emissions are so complex that at present the research is challenging. Up to now, no pheromones have been conclusively identified, but a strong contender for the first identified human pheromone is some elusive compound in the armpit of women. Apparently this unidentified pheromone causes menstrual synchrony in females living in close quarters. It has been speculated that its identification could potentially open the door to sniffable contraceptives ².

Chemical basis for olfactory communication In animal species, recognition between individuals is an essential requirement for any kind of further interaction. Recognition between mother and newborn is a fundamental behavioral interaction that is worth systematic investigation. The emotional relationship between a mother and her newborn begins with mutual recognition, which starts during gestation and continues through birth, augmented by body contact and lactation. Imprinting takes place through visual, auditory, and olfactory learning, which occurs very early during the so-called “critical period”.

Consequently, from beginning of pregnancy olfaction seems to represent an Ariadne's thread that permits the infant after birth to find its mother³.

Pheromones regulate reproductive behavior in many animal species. Once released in the environment, through urine or glandular secretions, these volatile substances reach other individuals of the same species, signaling, for instance, mating availability and strengthening ties between mother and offspring, as well as regulating social relationships⁴. In non-human vertebrate species, pheromones are detected by a specific sensory apparatus, the vomeronasal system, composed of a peripheral organ located at the base of the nasal septum, the vomeronasal nerve, and a nerve center, the accessory olfactory bulb. The vomeronasal system is completely separated and independent from the main olfactory system⁵. It is triggered by a different class of volatile substances and is present in many reptiles and in almost all mammals, but absent in fishes and birds, even if they possess a main olfactory system.

Primates, long considered functionally non-microsomatic, were previously thought to show complex olfactory communication only in prosimians and in some New World monkeys. Now it is well established that even higher primates use pheromones to recognize con-specific individuals and for territorial marking⁶⁻⁸. Furthermore, protein-pheromone complexes – present in the secretions released by scent-marking of some non-human primates – were recently shown to activate vomeronasal receptors particularly for sexually related behaviors and intra-specific identification of individuals⁹⁻¹².

Formerly, it was widely held that the human vomeronasal organ was vestigial and even the existence of pheromonal communication in humans was contested. However, support for a role of pheromones in human behavior came from several observational studies (i.e. synchronization of the menstrual cycle¹³), but this role was often denied because in the absence of a sufficient neuro-anatomic basis for such a complex behavior.

Recently, a new class of olfactory receptors (trace amino-associated receptors, TAAR) was discovered in the olfactory epithelium of mice¹⁴. Genes similar to those responsible for the control of these receptors in mice

have been identified in humans and fishes. These data suggest a great evolutionary conservation of these genes and lend support to the hypothesis that the putative human pheromonal response is mediated by receptors located in the main olfactory system.

The putative human pheromones are steroids present in the secretions of exocrine glands¹⁵⁻²⁰. Estrogen derivatives are present in females (the so-called “copulins” - mixtures of aliphatic acids such as acetic, propionic, butyric, isovaleric, and isocaproic acid with estratetraenol), and androgen derivatives are present in males (androstanol, androstene, and androstadienone). Recent studies concerning the most volatile compounds of human sweat²¹⁻²³ have shown that the characteristic odor produced by the para-axillary region is due to the presence of volatile C6-C11 acids, the most abundant being *E*-3-methyl-2-hexenoic acid (*E*-3M2H).

Mother-child identification Human chemical signals may also play a role in offspring identification. Odor cues from newborns are absolutely salient to their mothers²⁴. Mothers are able to distinguish the odor of their own newborn baby from that of other newborns²⁵. Experiments also have demonstrated that adults can even recognize gender and individuality of non-related children²⁶. Thus, body odors can provide humans with important information about the individual identity of their offspring²⁷⁻³².

On the other hand, children usually prefer parts of clothes that were in contact with the axilla and worn by their own mothers to clothes worn by other mothers²⁵. Therefore, chemical signals seem to have a fundamental role in the mechanism of mother-child identification^{30,33}. Breast-feed versus bottle-feed infants show different reactions to maternal odors. Breast-feed infants are exposed to salient maternal odors and rapidly become familiarized with their mothers' unique olfactory signature³⁴. Apparently, orientation to lactating-breast odors is an inborn adaptive response of newborns³⁵.

It seems an inescapable conclusion that naturally occurring odors play an important role in mediating infant behavior. Even fetal olfactory learning seems to occur and breast odors from the mother exert a pheromone-like effect at the newborn's first attempt to locate the nipple. Newborns are generally

responsive to breast odors produced by lactating women³³. Olfactory recognition may be implicated in the early stages of the mother-infant attachment process, when the newborns learn to recognize their own mother's unique odor signature: this process is possibly made easier by the high norepinephrine release and the arousal of the *locus coeruleus* at birth³⁶. Human infants are responsive to maternal odors beginning shortly after birth. They show an attraction to amniotic fluid odor that may reflect fetal exposure to that substance (i.e. prenatal olfactory learning³⁷). Moreover, human amniotic fluid seems to carry individualized odor properties, which are hypothesized to initiate parent-infant interactions³⁸.

Not only chemical signals: some socio-biological remarks It is clear that, as any other organism, humans are subject to invisible but potentially irresistible influences of metabolic materials on our muscle, motive and motor actions, both directly and indirectly^{39,40}.

Obviously, mutual recognition among organisms is related to many aspects of the personal profile, both of metabolic-material biological similarity and morphologic-motor ecological familiarity⁴¹.

Mothers recognize the sounds of their own babies' chortles and cries, and may differentiate among their causes, from tiredness to hunger, from pain to scare. Yet mothers may pause for a moment to listen harder to cries from an unseen child that are similar enough to their own babies' cries to require more attention before discounting and dismissing them as indeed being from someone else baby⁴².

Yet it is appreciated that the cry from any newborn can cause agitation and distress in any mother. Perhaps this reflects the process of acquiring maternal memories: that is, at the time a mother has a newborn, she does not yet know her babies cry well enough to discriminate with certainty. Moreover, mothers of newborns are in states of hormonally heightened metabolic arousal, and are thus readily responsive to both biologically and ecologically conditioned cues⁴³.

Immediacy of recognition of a simple similar-enough sound in a noisy place, such as a familiar name, is well enough known (the "cocktail party syndrome"), even if that name was not in fact said, and so what was heard was only 'similar enough' to seem 'familiar enough'.

The connection among all aspects of recognition is surely not sets of discrete 'signals' but, instead, a composition of several sorts of similarities and familiarities, including biochemical features. Probably these features serve to substantiate and sustain mutual recognition⁴⁴.

Volatile compounds behind mother recognition In our recent study³ we hypothesized that women probably develop a volatile profile, through pregnancy and childbirth, that enables identification of the mother by the newborn. The aim was to understand, through an analytical approach, how the volatile pattern of pregnant women changes during pregnancy and, consequently, to verify the effective role played by volatile chemical signals in the mechanism of mother-infant recognition.

We identified the volatile compounds in sweat patch samples collected from the para-axillary and nipple-areola regions of women during pregnancy and after childbirth. Results showed that during pregnancy women developed a distinctive pattern of five volatile compounds common to the para-axillary and nipple-areola regions (1-dodecanol, 1-1'-oxybis octane, isocurcumenol, α -hexyl-cinnamic aldehyde, and isopropyl myristate).

Hypothetically, the differentiation of the olfactory pattern among pregnant women helps newborns to recognize their own mother and distinguish her from other individuals. At the time of birth and during the first weeks of life, the distinctive olfactory pattern of the para-axillary area might be useful to newborns to recognize and distinguish their mother, whereas the characteristic pattern of the nipple-areola region is probably useful as a guide to nourishment.

Future challenges These recent results show the effectiveness of the methodology used. Through the collection and analysis of the secretion released at the level of the para-axillary area and in the nipple areola region, it is possible to investigate the volatile compounds in a proper way. Up to now these phenomena have often been investigated with inadequate methodologies and, as a consequence, the role of volatile compounds as regulators of mother-infant recognition has been underestimated. Moreover, recently developed technical instruments and procedures – as Solid Phase Micro-Extraction (SPME), Dynamic Head-Space Extraction (DHE), and Maldi TOF/TOF – in addition to the classic ones – as Gas

Chromatography-Mass Spectrometry (GC-MS) and Liquid Chromatography-Mass Spectrometry (LC-MS) – now allow investigators to characterize volatile and non-volatile compounds with high reliability⁴⁵.

The study of the mechanism of mother-infant recognition is important not only for the acquisition of new knowledge concerning the emission of signal molecules essential for mother-child identification, but also for its clear practical consequences. This information can be helpful for setting up the proper conditions to establish solid mother-child bonding. It can indicate the behavior and conduct to maintain during gestation and the initiation period of life of the newborn. Therefore an understanding of the mechanisms of newborns recognition of their mother could have practical health implications.

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